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Review

Review of efficacy trials of HIV-1/AIDS vaccines and regulatory lessons learned
A review from a regulatory perspective

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ABSTRACT

The clinical development of prophylactic HIV-1/AIDS vaccines is confounded by numerous scientific challenges and these in turn result in challenges to regulators reviewing clinical trial applications (CTAs). The search for an HIV-1/AIDS vaccine will only succeed through the conduct of well-designed, well-conducted and well-controlled human efficacy studies. This review summarizes relevant context in which HIV vaccines are being investigated and the six completed efficacy trials of various candidate vaccines and regimens, as well as the lessons learned from them relevant to regulatory evaluation. A companion review focuses on the scientific challenges regulators face and summarizes some current candidates in development. The lessons learned from the completed efficacy trials will enable the development of better designed, potentially more efficient efficacy trials in future. This summary, supported by the World Health Organization (WHO), is unique in that it is meant to aid regulators in understanding the valuable lessons gained from experience in the field to date.

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1. Introduction – scope and purpose of review

Prophylactic Human Immunodeficiency Virus Type 1[1] (HIV) vaccine clinical trials, including efficacy trials, are being conducted in countries worldwide. Many of the countries hardest hit by the HIV/AIDS (Acquired Immunodeficiency Syndrome) epidemic are in sub-Saharan Africa or Asia and most of those are in low- and middle-income countries. The World Health Organization (WHO) has the responsibility to aid countries in enhancing regulatory capacity and undertook this review to frame scientific issues relevant to the development of HIV/AIDS vaccines, in the context of regulatory review and evaluation. The purpose of this is to facilitate the development process and sound regulation of HIV/AIDS clinical trials, as well as to provide technical support to regulators. Currently, the development of experimental preventive HIV/AIDS vaccines includes a wide variety of innovative and complex approaches without precedent in other licensed vaccines. Lessons learned from completed clinical trials using such new approaches, e.g., vectored vaccines or heterologous prime-boost immunization, can be informative to regulators as they face review of future clinical trial applications (CTAs) as the search for a safe, efficacious, globally useful HIV/AIDS vaccine continues. WHO has been facilitating the research and development of HIV/AIDS vaccines and providing support to strengthen the technical capacity of regulators in assessing CTAs for HIV/AIDS vaccines. This review is intended to summarize some of those lessons learned relevant to regulators and regulatory evaluation.

In response to requests to WHO from regulators, vaccine developers, and manufacturers on HIV/AIDS vaccines, a review of six completed clinical efficacy trials conducted with HIV/AIDS vaccine candidates was undertaken by WHO in order to provide a comprehensive overview of the main challenges and lessons learned during the preparation, conduct, monitoring and data interpretation of these trials. Key outcomes of the review are summarized in this article with the aim of making a compilation of the knowledge about HIV/AIDS vaccine development available to regulators, manufacturers, academia, funding agencies and other relevant stakeholders engaged in various aspects of HIV/AIDS vaccine development and regulatory evaluation. A companion review summarizes the most advanced candidate vaccines likely to move forward to future efficacy trials and the scientific challenges that remain in the field of HIV/AIDS vaccines, which present regulatory challenges.

2. HIV epidemiology

According to the UNAIDS 2014 Gap Report [1] 39 million people are living with HIV today. Of these, 3.2 million are children under the age of 15 and 18.5 million of those are women or girls. The majority of these cases are in Sub-Saharan Africa, where the estimates are 24.7 million. There are an additional 4.8 million cases in South and South-East Asia.

Due to the way that statistics are gathered and calculated, adolescents aged 15 years of age and older are included in the estimates for adult statistics. However, adolescence is a period of tremendous increased risk for acquiring HIV infection as sexual activity is initiated. Thus, this age group is undeniably the target population for a successful vaccine; however, there are challenges to conducting studies in this vulnerable population.

The primary route of transmission of HIV remains heterosexual transmission. However, intravenous drug use (IDU or PWID – people who inject drugs), mother to child transmission (MTCT), and men who have sex with men (MSM) remain important contributors to the transmission rates around the world.

Although in many countries, transmission rates are slowing, the existing prevention modalities have not completely controlled the epidemic. Improvements in prevention modalities, including treatment of infected individuals to reduce transmission rates to uninfected partners, are increasingly prevalent and more widely in use. Their impact is being seen in the reduction in transmission rates; however, these improvements have not eliminated the epidemic in any one country. Thus, the need for a vaccine that can prevent acquisition of HIV infection is still undeniable. No thought can be given to eliminating the disease of AIDS without a prophylactic vaccination programme.

3. Need for a vaccine in context of other prevention strategies

Considerable progress [2] has been made in the past decade on prevention modalities; male circumcision, treatment of the HIV-infected partner to prevent infection in the sero-discordant partner, post-exposure prophylaxis (PEP), pre-exposure prophylaxis (PrEP), and microbicides. These more recent prevention strategies add to the existing strategies of blood supply safety, condom use, access to sterile injection equipment, delay to initiation of sexual activity or abstinence, monogamy between known uninfected partners, voluntary testing and counseling, and knowledge, including knowing one's current infection status and how to protect oneself.

It has been questioned whether an HIV/AIDS vaccine is even feasible. Given the ever increasing number of successful prevention strategies, some question the effort towards vaccine development. While it is true that in some countries, declines in HIV incidence have thankfully been achieved with existing and new prevention strategies; in other countries, the epidemic is still increasing. In no one country has the epidemic been controlled or eliminated. Successful vaccines have resulted in the control and in some cases elimination of many infectious diseases, including diphtheria, measles and polio (among many others). The smallpox vaccine permitted eradication on a global scale of that disease. Thus, it is likely that only with the development of a successful HIV/AIDS vaccine can the control of this epidemic be conceivable. Despite numerous challenges, discussed in the companion review, the effort to develop an HIV/AIDS vaccine should not be abandoned. Given that individuals, once infected, remain infected for the rest of their lives and can potentially transmit infection to others throughout this time (although effective anti-retroviral treatment that controls viral load leads to successful interruption of transmission as long as the treated individual remains adherent and does not develop drug-resistance resulting in loss of viral load control), disease control in a public health sense can only be achieved with a prevention modality (or combination) that achieves extremely high effectiveness at a population level. Vaccines are the only prevention modality that do not rely on sustained behavior-modification, which are known to be able to achieve this high degree of effectiveness. Although initial HIV/AIDS vaccines may not be so highly effective, efforts to develop such highly effective vaccines must continue. Even partially effective HIV/AIDS vaccines may have a positive impact on a population level [3].

4. History of clinical development of HIV/AIDS vaccines

The clinical development of candidate HIV/AIDS vaccines began in the late 1980s. Since that time, more than 100 candidate

[1] Throughout this review, the terms HIV or HIV/AIDS will be used and refer to HIV-1. To our knowledge, few, if any efforts are ongoing in clinical development of HIV-2 vaccines, so this review is strictly confined to HIV-1.

prophylactic vaccines have been tested in Phase 1 clinical trials, conducted in countries worldwide. Some candidates have moved forward to Phase 2a studies, but few have advanced to the stage of Phase 2b or Phase 3 efficacy studies [4]. Among those that have been clinically tested for efficacy, only one candidate regimen has so far shown modest efficacy. The remainder have failed to demonstrate efficacy and in one case, there was a trend towards possible enhanced risk of acquisition of HIV infection, acquired through risk behavior, in those vaccinated.

There are numerous scientific challenges to the development of an HIV vaccine, as highlighted in the companion review. These challenges have so far vexed the HIV vaccine development field. As the risk for failure involved in HIV vaccine development is so considerable, industry has been reluctant to invest the tremendous amounts of resources needed for such development without some mechanisms to share the risk. As a consequence, public–private partnerships, public clinical trial networks, and public funding have been central to progress in the field.

4.1. International collaborative efforts to facilitate the clinical development of HIV/AIDS vaccines

Due to the difficult challenges for developing HIV/AIDS vaccines, several public–private partnerships and clinical trials networks have taken up these challenges. The field has recognized that no one individual or organization is capable of overcoming so many hurdles and that only through international collaborative efforts may these challenges be faced. Listed below are several active participants in these international collaborative efforts, including donors, clinical trial networks, non-profit organizations, advocacy groups, and other important parties, including the WHO. For more information on each, where appropriate, weblinks are provided. Important players in the field include:

- **The WHO-UNAIDS HIV Vaccine Initiative and Other Relevant WHO Initiatives**
  The WHO has played a critical role in advising countries on issues relevant to the development of HIV/AIDS vaccines. The WHO has held a number of consultations to facilitate information sharing, to reach consensus, and to develop appropriate guidance on topics covering ethics, inclusion of adolescents and women in clinical trials, vaccine quality issues, non-clinical issues and clinical issues important to HIV vaccines.
  In addition to the efforts by the WHO, there is a WHO standing AIDS Vaccine Advisory Committee (VAC), which has assisted countries in the review of proposed clinical trials. The WHO supports the African Vaccine Regulatory Forum (AVAREF), which holds ongoing discussions that assist regulators across Africa in the consideration of regulatory issues on HIV, malaria, tuberculosis, and other vaccines of relevance to the continent. The WHO supports the Developing Country Vaccine Regulatory Network (DCVRN), which likewise meets regularly to discuss regulatory issues with regards to vaccines of relevance to the 11 countries that participate in this network. The purpose of the DCVRN is to contribute to the strengthening of the National Regulatory Authorities (NRA) in low- and middle-income countries where vaccines are manufactured, particularly in the area of authorization and evaluation of vaccine clinical trials. WHO-UNAIDS also developed the African AIDS Vaccine Programme (AAVP), whose secretariat was transitioned to Uganda in 2010.
  Finally, the WHO has several Collaborating Centers, some of which have clinical trials evaluation expertise and all of which have laboratory expertise relevant to the manufacture of and analytics for vaccines. More information may be obtained from http://www.who.int/collaboratingcentres/en/ (accessed 11/17/2014).
- **The U.S. National Institutes of Health's (NIH) Division of AIDS (DAIDS), the HIV Vaccine Trials Network (HVTN), Other DAIDS-Funded Clinical Trials Networks (CHAVI-ID, HPTN, IMPAACT), and the Vaccine Research Center**
  Undoubtedly, the largest funder of HIV vaccine research has been the U.S. National Institutes of Health (NIH). In addition to funding academic research as well as funding development by biotechnology companies and large pharma/vaccine companies, the Division of AIDS (DAIDS) at the NIH funds several clinical trials networks, which perform clinical trials of candidate HIV vaccines and other interventions developed by academicians, as well as small and large industry. Among these, the greatest number of vaccine trials has been conducted by the HIV Vaccine Trials Network (HVTN) and its predecessor, the AIDS Vaccine Evaluation Group (AVEG). The HVTN has clinical trials sites on most continents. In addition, DAIDS funds the HIV Prevention Trials Network (HPTN), which has performed important studies on microbicides, post-exposure prophylaxis (PEP), pre-exposure prophylaxis (PrEP), and other prevention interventions. Studies undertaken with a focus on mother to child transmission (MTCT) or on vaccination or prevention in infants, children, or adolescents are the subject of the DAIDS' funded International Maternal, Pediatric, and Adolescent AIDS Trials Network (IMPAACT). More recently, DAIDS funded two Centers for HIV/AIDS Vaccine Immunology and Immunogen Design (CHAVI-ID), which will also be responsible in conducting clinical trials of immunogens they have designed. Finally, but importantly, the intramural NIH Vaccine Research Center conducts primary research, as well as preclinical and clinical research with an aim to develop a successful HIV/AIDS vaccine. Some of this research will be reviewed below, particularly the regimen tested for efficacy in HVTN 505.
- **International AIDS Vaccine Initiative (IAVI)**
  Another donor, which is a global non-profit organization, is the International AIDS Vaccine Initiative (IAVI, www.iavi.org). This organization supports the development of a number of potential HIV/AIDS vaccine candidates and has clinical trial sites and laboratories in several countries.
- **The U.S. Military HIV Research Program (USMHRP)**
  The U.S. military has a long history in combatting tropical diseases and in vaccine development. The USMHRP (http://www.hivresearch.org/home.php, accessed 8/19/2013) has several clinical trials sites with which they work in multiple countries worldwide, as well as active laboratories and vaccine development efforts.
- **P5 – the Poxvirus-Protein Public–Private Partnership**
  As an outcome of the RV144 study, a Poxvirus-Protein Public–Private Partnership (or P5; http://vaccineenterprise.org/content/P5Partnership) was organized with the purpose of extending the results of that critical efficacy study. The P5 is a collaboration between industry (Sanofi Pasteur, Novartis, and GlaxoSmithKline), the U.S. government (DAIDS and USMHRP), the HVTN, and the Bill & Melinda Gates Foundation.
- **The Bill & Melinda Gates Foundation (BMGF)**
  Another funder of increasing and significant importance in the arena of global health in general and in the development of vaccines for several diseases of global importance, including HIV/AIDS, in particular, is the Bill & Melinda Gates Foundation. (http://www.gatesfoundation.org/, Accessed 2/28/2014) They support basic and clinical research.
- **The AIDS Vaccine Advocacy Coalition (AVAC)**
  An advocacy coalition that has played an important role in public education, public policy and promotion of issues relevant

to the development of HIV vaccines is the AIDS Vaccine Advocacy Coalition (AVAC). (http://www.avac.org/ Accessed 2/28/2014)

- **The Enterprise**
  The Global HIV Vaccine Enterprise was established at the suggestion of a number of notable scientists from around the globe, active in the HIV/AIDS vaccine field. It was recognized that the problem of HIV/AIDS vaccine development was so significant that no one organization or individual would be apt to solve the problem working in isolation and that a large enterprise was needed to coordinate and streamline global efforts. (http://vaccineenterprise.org/ Accessed 2/28/2014)
- **EuroVacc Foundation**
  Within Europe, one notable organization focused on the development of HIV/AIDS vaccines is EuroVacc Foundation, a non-profit organization. (http://www.eurovacc.org/ Accessed 2/28/2014) The Foundation supports basic, preclinical, and clinical research.
- **Agence Nationale de Recherche de la SIDA et les hepatitis virales (ANRS)**
  French research agency that has developed and tested HIV/AIDS vaccine candidates. (http://www.anrs.fr/, accessed 8/11/2015)
- **Other National Initiatives, Advocates**
  The Ministry of Public Health, Thailand; TAVEG (Thai AIDS Vaccine Evaluation Group); SAAVI (http://www.saavi.org.za/, accessed 8/17/2013); KAVI (http://www.kaviuon.org/about_us.php, accessed 8/17/2013); and Canadian HIV Vaccine Initiative (CHVI, http://chvi-icvv.gc.ca/index-eng.htmlaccessed 11/25/2013).

4.2. More than 100 HIV/AIDS vaccine candidates have been tested clinically

It is not for the lack of clinical candidates that so few have advanced to efficacy trials. In fact, HIV vaccine development, more than any other vaccine field, has driven the novelty of approaches to immunogen design, delivery, and complexity. Initial candidates were simple subunit approaches, particularly based on the envelope gene (*env*) in the forms of full length (gp160) and the cleaved, soluble portion (gp120) or various truncated forms (some soluble, some transmembrane-spanning, gp140, gp145, or gp150). In addition to alum adjuvant, multiple novel adjuvants have been tested with these protein immunogens. After testing simple subunit vaccines, more complex designs were tried, including peptides, lipopeptides, and vectors expressing various genes of HIV. Vectors range from DNA plasmids, non-replicating viral vectors, replicating viral vectors and bacterial vectors. Among the non-replicating viral vectors are those that were deliberately made to be non-replicating such as the Adenoviral vectors, with gene deletions that preclude replication except in complementing cell lines, and those that do not replicate in humans, such as several of the poxvirus vectors, but which replicate in other species' cells (e.g., chick embryo fibroblasts). Summaries and lists of prior candidates are published elsewhere (Jordan report; http://www.niaid.nih.gov/topics/vaccines/Pages/Jordan2012.aspx) [5].

One important feature of immunogen delivery that arose in the effort to improve *in vivo* potency of vectored HIV/AIDS vaccines was the heterologous prime-boost concept, in which one or more DNA plasmids or one or more viral vectors serve for the initial priming doses followed by, respectively, a or another viral vector(s) or protein subunit(s), which serve as the subsequent boosting immunogen(s). Other diverse methods for immunogen delivery included the use of novel delivery devices, such as the Biojector, or inclusion of electroporation or facilitators (e.g., bupivacaine) with DNA plasmid delivery to facilitate uptake *in vivo*.

The diversity of approaches that have been tested pre-clinically and clinically for HIV vaccine candidates is considerable, but many Phase 1 studies failed to demonstrate the promise of the approach that the pre-clinical data suggested and development of most candidates has been abandoned. This illustrates the limitations of the pre-clinical animal models, which as yet are not predictive of clinical success. It would seem that the lack of immunogenicity in an animal model may be predictive of a lack of clinical activity, but potent immunogenicity, or even evidence of efficacy, in animal models has not fully been evidenced with clinical promise. Thus, human clinical trials are presently the only way to truly determine whether an HIV vaccine candidate will have activity or efficacy in humans. Regulators should appreciate this fact when considering what amount and types of preclinical data to expect in review of a CTA.

As a consequence of lack of significant immunogenicity in Phase 1 (or in rare cases, adverse safety signals), only a limited number of approaches have emerged to advanced clinical development. Those that have been tested clinically for efficacy are described below. As stated above, the important lesson in the HIV vaccine field is that the pre-clinical models, though useful tools for many purposes, have yet to achieve the ability to predict which vaccine(s) will be efficacious in humans, with the most potent of approaches that have yielded promising efficacy data in animal models having failed to recapitulate this promise in clinical efficacy testing. Thus, it must be recognized that until the clinical efficacy trials are performed and the results become known, no one can know whether a particular immunologically-promising candidate vaccine, regimen, or design will yield clinical efficacy. However, work must continue to improve upon the animal models because human efficacy studies are expensive in many terms of various types of resources, particularly human resources, but also financial resources and materials and laboratory capacity. No one wishes to place human trial subjects at risk with no possibility of benefit, so some means must be used to identify the most promising candidates to move forward. This remains one of the biggest scientific hurdles in the HIV vaccine field.

4.3. Phase 2b or Phase 3 efficacy studies

This section of the review describes the six clinical efficacy trials that have been completed to date and their results, as well as lessons learned relevant to regulators. Each study was performed because promising early pre-clinical and clinical data supported moving forward into advanced development. Though essentially in every case, the exact promise of those early clinical data (and pre-clinical data) were controversial among those working in the field. These controversies reflect the scientific uncertainties in the field. Each study was justified based on certain existing data at the time they were initiated. These data include safety and immunogenicity data from earlier human clinical trials, as well as efficacy (challenge–protection) data in some non-human primate models. In point of fact, perhaps the most controversial of these efficacy studies was the RV144 study, which, as it turned out, was the only study to demonstrate modest efficacy, while other candidates thought to be the most promising at any given time had no efficacy in humans. This underscores the scientific challenges to developing an effective HIV/AIDS vaccine and the uncertainties that exist within the field and why we must be humble and agnostic as scientists when considering CTAs.

Nonetheless, regardless of the ultimate outcomes of the studies, each was conducted in compliance with Good Clinical Practices (GCP) and appropriate ethical standards. Each was well-performed,

gave definitive and conclusive results and demonstrated that HIV/AIDS vaccine efficacy studies were feasible and could be conducted with good recruitment and retention. Positive lessons were learned in the conduct of each study and those lessons were built upon in subsequent studies.

The lack of clear-cut and obvious scientific criteria to support advancement into efficacy testing is a difficulty that regulators must be cognizant of and grapple with in their review of CTAs for efficacy studies. Experts in the field are uncertain and disagree among themselves about which candidates warrant advancement. Thus, regulators should recognize that they can hardly be expected to know for certain, when foremost authorities on the subject do not.

One should not become too overly concerned with the nomenclature of Phase 2b or Phase 3, or pilot or pivotal, when considering HIV/AIDS vaccine efficacy studies. The important regulatory consideration, rather than this nomenclature, is whether or not the efficacy study as designed would support licensure or registration, as a sole efficacy study or as one of two or more efficacy studies of that vaccine candidate or regimen. The important facet for a licensure trial is the power the study has to credibly establish efficacy and the primary claims for labeling of that vaccine candidate or regimen. Another important regulatory consideration would be, in terms of correlates of protection, whether the study would be designed to be hypothesis-generating or hypothesis-testing. However, correlates of protection are not necessarily required for licensure of an efficacious vaccine, although they greatly facilitate vaccine development. So, whatever the efficacy study is called, its design should be considered in review of the CTA, as to whether the regulator would consider it as means to identify hypotheses to be later tested in a pivotal, licensure-supporting trial, or whether it tests appropriate licensure-supporting hypotheses and is itself sufficient to support licensure (registration) decisions. A clear understanding between regulators and applicant(s) should be in place when the regulators allow the trial to proceed, to prevent being faced with too few data upon which to make a meaningful licensure (registration) decision at the study's completion.

To date, none of the efficacy studies that have been conducted have supported the licensure of an HIV/AIDS vaccine regimen solely on their own merit. However, it will be noted below whether the intent of the studies at their outset was to be licensure-supporting (pivotal) or pilot (Phase 2b) studies to guide further clinical development.

4.3.1. VAX004

The VAX004 study was conducted by VaxGen from 1998 to 2003 in North America and the Netherlands, primarily in HIV-uninfected MSM, but also included high-risk heterosexual women. The candidate vaccine was a bivalent clade B gp120 subunit vaccine adjuvanted with alum. Individuals using intravenous drugs (IDU or PWID) were not enrolled in this study. This Phase 3 study was performed with intention to support licensure, in conjunction with the VAX003 study described below, which enrolled IDU or PWID. Individuals were randomized 2:1 to receive vaccine (300 mcg each of MN gp120 and GNE8 gp120 adsorbed onto 600 mcg alum) or placebo (alum). Vaccinations were given at months 0, 1, 6, 12, 18, 24 and 30, with the final study visit at month 36. HIV status was assessed by standard HIV-1 ELISA and confirmatory immunoblot. Time of infection was estimated to be the midpoint between the last negative ELISA and immunoblot and the first positive one. HIV plasma RNA was also measured. The date of infection may have been taken as the first date when a positive RNA sample was measured. For volunteers who became infected on the study (from their own risk behavior despite extensive risk reduction counseling), CD4 counts and viral load (plasma RNA) were measured at <1, 1, 2, 4, 8, 12, 16, 20, and 24 months post-diagnosis. Questionnaires on self-reported risk behaviors were performed at baseline and every six months. Viruses from infected individuals were sequenced. All isolates except 1 were clade B. The exception was clade C. Immunogenicity was evaluated by neutralization against MN and binding antibodies by five indirect ELISAs to MN/GNE8 gp120 mixture, GNE8 V2, MN V2, GNE8 V3, and MN V3. A further two competitive ELISAs were conducted to measure blocking antibodies against either MN or GNE8, with soluble CD4. These analyses were performed two weeks after the last immunization before infection occurred and two weeks after every immunization on a random 5% subset.

The primary endpoint of the study was Vaccine Efficacy [VE; $(1 - \text{relative risk of infection}) \times 100$]. The time to HIV infection was grouped by six month intervals. The study was designed to have 90% power to reject a null hypothesis of VE $\leq$ 30% if the true VE was $\geq$60%. Secondary endpoints related to viral loads before initiation of anti-retroviral therapy (ART) and time-to-initiation of ART between the two study arms. Exploratory analyses were performed on demographic factors, such as age ($\leq$30 or >30), race (white, black, Hispanic, Asian, or other and white vs. non-white), gender, education (less than a college degree or college or graduate degree), and baseline behavioral risk (high, medium, low).

Between June 1998 and October 1999, 7185 subjects were screened and 5417 eligible subjects enrolled (5108 men and 309 women). Despite negative baseline HIV antibody screening, 14 subjects were subsequently found to be HIV-infected at baseline and were excluded from efficacy analyses. 11 of these were enrolled into the vaccinee group and three into the placebo group. 368 volunteers became infected on the study (through their risk behavior) for an annualized rate of 2.6% (2.7% in men and 0.8% in women). Only six women became infected during the study.

Controversy to initiate this study arose when, in 1994, in an open public meeting at the National Institutes of Health (NIH), the NIH decided not to advance either VaxGen's or Chiron/Biocine's gp120 candidate vaccines into efficacy testing because, while they each generated strong binding antibodies and strain-specific neutralizing antibodies, they did not generate antibodies that could broadly neutralize multiple clade B strains by the latest assay methods. Because of the variability of HIV, concern arose over the utility of strain-specific neutralization to protect against the broad array of viruses that would be encountered by participants in an efficacy trial (through their high risk behaviors). VaxGen, nonetheless, spent the subsequent years gathering funding and resources to initiate both the VAX004 and VAX003 studies (to be described below) without significant support from NIH funding.

Some information in this section and subsequent sub-sections has been taken from the publication of the efficacy study results in the article by the rgp120 HIV Vaccine Study Group [6]. Information has been paraphrased or sometimes directly quoted.

4.3.1.1. Safety. The vaccinations were stated to be well-tolerated. Most common events were mild to moderate in the first three days following vaccination. Rates of local symptoms were higher in vaccinees than placebo-recipients, as follows, as reported on one of 14 days following vaccination:

	Local edema	Induration	Subcutaneous nodule
Vaccinees	36%	29%	21%
Placebo recipients	17%	15%	12%

It was stated that there were no other major differences in rates or types of reported adverse experiences between groups.

4.3.1.2. Immunogenicity. All vaccinees developed antibodies against gp120. Vaccinees with higher antibodies that were MN CD4-blocking, GNE8 CD4-blocking, and MN neutralizing tended to have a lower rate of HIV infection. Virus sequences were analyzed to determine whether there was a differential impact of those that contained the same V3 domain sequences as the vaccine (GPGRAF) and those that did not. This selective pressure has also been referred to as a "sieving effect." Overall, there was no evidence of this effect with 0% VE among those infected with GPGRAF-containing and 19% VE in those infected with viruses that did not contain that sequence. However, there was a non-significant trend to an estimated VE of 73% in non-whites with viruses with the GPGRAF sequence (95% CI: 35–88%) vs. an estimated VE of 24% in those infected with viruses without this sequence (95% CI: −59 to 63%) for a p-value of 0.077.

4.3.1.3. Efficacy. Efficacy was not observed [VE 6% (95% CI −17 to 24%), $p = 0.59$]. Kaplan–Meier curves revealed equivalent approximately constant rates of infection over 36 months. Pre-ART viral loads were similar in both groups ($p = 0.81$). Rates of initiation of ART were similar between groups ($p = 0.61$). There were no interactions noted for efficacy with age, gender, or education levels. However, for baseline risk levels, VE varied significantly ($p = 0.041$) with VE decreasing in the highest risk group and by race ($p = 0.007$) with lower VE in Caucasians ("whites") than in other racial groups ("non-whites").

4.3.1.4. Lessons learned relevant to regulatory evaluation. Controversy was raised by discordant announcements in the press over the outcome of the study because of the sub-group analyses that suggested that while there was no efficacy in the overall population, there appeared to be efficacy in "non-whites." The press either reported that the study demonstrated efficacy or more correctly, that it did not. However, a lesson learned from this experience relevant to regulators is the need for sponsors to be clear in the statistical analysis plan about when to continue to analyze sub-groups when efficacy overall was not seen and whether these additional analyses will be hypothesis-testing or hypothesis-generating. The study sponsors had prospectively planned the sub-group analyses, but it may have been a regulatory expectation that claims would not be made on the basis of these tertiary analyses, when the primary analysis did not refute the null hypothesis. Thus, regulators should clearly articulate their expectations when reviewing a sponsor's statistical analysis plan, in regards to claims made regarding the study's outcome. The sub-group analyses were appropriate as hypothesis-generating, given the failure of the primary analysis. If the primary analysis had been positive, then claims might be considered on the basis of the secondary analyses or possibly even tertiary analyses. However, when the primary analysis is negative, caution in interpreting secondary and tertiary analyses must be taken.

The other controversial statistical issue raised with the reporting of study results came from the lack of statistical adjustments taken as a result of the multiplicity of analyses performed. Statistical adjustments, like the Fleming–O'Brien approach, should be incorporated into the statistical analysis plan and reports of statistical significance should note whether or not the p-value has been adjusted, accordingly.

4.3.2. VAX003

The VAX003 study was conducted by VaxGen and the Ministry of Public Health of Thailand from 1999 to 2003, in Thailand in intravenous drug users (IDU or PWID). This Phase 3 trial was conducted to provide additional evidence to support licensure by having two well-controlled efficacy studies in different risk populations. However, as the circulating strains in Thailand differ from those in North America, the bivalent vaccine contained not only the clade B MN strain (300 mcg), but also the clade E (recombinant A/E clade) A244 strain gp120 (300 mcg), adjuvanted in alum (600 mcg). The placebo consisted solely of alum. The study was randomized 1:1 vaccinees to placebo-recipients. HIV-uninfected volunteers aged 20–60 who had used intravenous drugs in the past year and were being treated at one of 17 Bangkok methadone treatment clinics were enrolled. For females to enroll, they could not be pregnant or breast-feeding and had to agree to use contraception while on study to reduce possible risks to fetuses. To ensure comprehension of the trial procedures and what they were volunteering to do in the study, potential volunteers had to pass two written trial comprehension tests. Volunteers received extensive risk reduction counseling at study visits. Condoms and bleach to sterilize injection equipment were provided to volunteers free of charge.

Like the VAX004 study described above, self-reported behavioral risk questionnaires were administered at baseline and every six months during the study. Vaccinations were given at 0, 1, 6, 12, 18, 24, and 36 months. The HIV status was judged by ELISA and confirmatory immunoblotting requiring at least two non-gp120 bands to be positive. HIV plasma RNA was measured in individuals evidencing seroconversion to HIV infection. If RNA was negative, the date of infection was estimated to be the midpoint between the last negative and first positive ELISA/immunoblot. Otherwise, the date was taken as the first RNA positive visit. Volunteers who became infected (by their own risk behavior) on study were followed at <1, 1, 2, 4, 8, 12, 16, 20, and 24 months for plasma RNA levels and CD4 and CD8 cell counts. Five immunogenicity ELISAs were performed for volunteers at either two weeks after the last immunization at which the volunteer remained HIV-uninfected or in a random subset of volunteers at each immunization and two weeks after the last immunization (10% of uninfected vaccinees and 1% of uninfected placebo-recipients). Viral sequences were obtained from infected individuals.

The primary study endpoint was acquisition of HIV infection. Secondary endpoints were safety and delay to disease progression, as judged by clinical endpoints (time to initiation of ART or AIDS-defining illness onset) and virological and cellular endpoints.

Some information in this section and subsequent sub-sections has been taken from the publication of the efficacy study results by Pitisuttithum et al. [7]. Information has been paraphrased or sometimes directly quoted.

4.3.2.1. Safety. Tenderness at the injection site was reported in 71% of vaccinees and 65% of placebo-recipients and did not increase with repeated inoculations. Of 414 Serious Adverse Events (SAEs) reported, the majority were accidental injury (128) and drug overdose (49), with sepsis occurring in 22 cases, with no differences in rates between groups. Likewise, among the 102 deaths, 38 were due to drug overdose, 17 to sepsis, 12 to accidental injury, and 8 to suicide, with no differences between groups.

4.3.2.2. Immunogenicity. There were no significant differences between infected vaccinees and randomly selected uninfected vaccinees in pre-infection antibodies (in infected vaccinees) or antibodies (in uninfected vaccinees) as measured by binding to gp120, A244 V2, A244 V3, blocking of A244 to CD4, or neutralization of MN. Antibodies were seen in all vaccinees in which they were measured and in no placebo recipients in which they were assessed ($n = 12$). Geometric mean neutralization titers after a complete primary immunization series (month 6.5) were 3972 and

remained at this level or slightly higher throughout the remaining boosting immunization periods, reaching as high as 5707 at month 12.5.

4.3.2.3. Efficacy. There were no differences in the time to HIV acquisition through risk behavior between the two groups, with 106/1267 vaccinees and 105/1260 placebo-recipients becoming infected on study. Efficacy was estimated at 0.1% with a 95% confidence interval of −30.8 to 23.8 and p = 0.99 (log-rank test). Likewise, no significant differences were seen in clinical endpoints in the infected, nor virological or cellular endpoints.

4.3.2.4. Lessons learned relevant to regulatory evaluation. Occasionally, people (including regulators) may pre-suppose that certain populations who are at highest risk of HIV infection by virtue of being a member of a marginalized community, e.g., IDU or PWID; are difficult, if not impossible, to recruit and retain in a clinical trial. VAX003 showed that it was feasible to successfully conduct a study in such a population and it required the political will of the Thai government to facilitate this happening in this study. In general, regulators know the political situation in their own countries much better than might applicants. Discussions on potential barriers to conduct a trial should follow review of the protocol, given the political situation in one's country or particular communities and should be considered between regulators and applicants to ensure the potential for a trial being approved, to be successfully conducted. Neither trial sponsors nor regulators benefit from a trial that fails to give a clear and credible answer. Working collaboratively, trials can be designed and conducted successfully, even when conducted in populations that might, on first impression, seem difficult or impossible to even consider. As the HIV/AIDS epidemic does affect many such marginalized populations, it would be a disservice at best and unethical at worst to ignore these populations when conducting HIV/AIDS vaccine efficacy studies.

4.3.3. STEP

This study was designed as a Phase 2b test-of-concept study and originally was designed to enroll only Ad5-seronegative subjects, but was later expanded to enroll Ad5-seropositive subjects. The concept being tested was whether a T-cell-inducing vaccine not expected to elicit antibodies of significance (e.g., against the envelope gene product, which was absent from the vaccine candidate) could have efficacy. The study was conducted by Merck Research Labs (http://www.merck.com/index.html, accessed 8/19/2013, USA) and by the HVTN in Australia, the Caribbean, and North and South America. The study product was a trivalent replication-incompetent Adenovirus type 5-vectored vaccine candidate expressing clade B HIV-1 *gag*, *pol*, and *nef* genes on a 0, 1, 6 month schedule at a dose of 1.5×10^{10} vector genomes (equivalent to a prior dose used of 3×10^{10} viral particles) in 1 mL. Enrollment in the seronegative population began in December 2004 and in July 2005, enrollment was expanded to Ad5-seropositive subjects with a target goal of 1500 of each. Seropositivity to Ad5 at baseline was defined as a reciprocal titer of ≥200. Participants were enrolled until March 2007. Trial subjects were 18–45 years of age. Different risk criteria permitted enrollment of men [MSM who had unprotected anal sex or anal sex with two or more partners in the prior six months, heterosexual men in Caribbean sites who had a history of particular STDs, exchanging sex for money or other commodities, use of crack cocaine, or multiple sexual partners (two or more in the prior six months)] or heterosexual women (who had unprotected sex with a partner known to be HIV-infected or an IDU, who used crack cocaine, or who exchanged sex for money or other commodities).

The endpoints of the study were safety and tolerability, as well as the following efficacy endpoints in the original trial population (Ad5-seronegative at baseline): acquisition of HIV infection and the average of viral load (plasma RNA) taken at two and three months post-diagnosis of infection. The per-protocol analysis considered all subjects who received their first two doses of study treatment to which they were randomly assigned and who were not diagnosed as infected until after week 12 on study, and did not violate the protocol. The MITT population included all those who received at least one study treatment and who were not HIV-infected at baseline, before randomization. Secondary endpoints were to evaluate safety, tolerability and efficacy in the entire study population regardless of baseline serostatus to Ad5 and to identify immune correlates of efficacy. Exploratory endpoints included interactions between the two primary efficacy endpoints and prognostic factors between demographics and efficacy (e.g., gender, baseline Ad5 serostatus, age, circumcision status in men, etc.). The study was designed and powered as an event-driven analysis, with the expectation of acquiring 50 endpoints (HIV infection) in each serostatus group, with a planned interim analysis at 30 endpoints. The sample size gave an 80% power to detect a ≥60% reduction in HIV acquisition or ≥1 $\log_{10}$ difference in set-point viral load in favor of the vaccinated group compared to the placebo-recipient group. Futility criteria were also established. At the interim analysis, if the 1-sided p-value was >0.5 for both efficacy endpoints, the vaccine would be declared ineffective and the study halted early for futility. Additional analyses were added because the study did unexpectedly meet the criteria for futility. These additional analyses are described in Buchbinder et al. [8]. The Data Safety Monitoring Board (DSMB) met in September 2007, reviewed unblinded data and recommended halting the study for futility.

Three thousand subjects were enrolled in the study, with 1515 of low Ad5-serostatus enrolled and 1485 Ad5-seropositive enrolled. More than 90% of vaccinees received all three inoculations. About 30% of the enrolled participants of low Ad5-serostatus were female and nearly 50% of Ad5-seropositive participants were female, but only one infection occurred in a woman, so most efficacy analyses were performed considering the infections in men only. Forty-five infections occurred during the study in the population that received at least one inoculation and who was not retrospectively found to be HIV-infected at baseline.

Like the earlier trials, this study met some controversy as well, because it was expanded into a larger population (the Ad5-seropositive population) before efficacy results in the "best-case scenario" (Ad5-low serostatus) population were known. Immunogenicity data from the Ad-5 seropositive population from earlier phase studies became available after the initial STEP study had begun. These data supported a robust cellular response to the vaccine insert genes in the face of pre-existing vector immunity, so the decision was made by the trial sponsors to expand the study on the basis of those immunogenicity results.

Some information in this section and subsequent sub-sections has been taken from the publication of the efficacy study results by Buchbinder et al. [8]. Information has been paraphrased or sometimes directly quoted.

4.3.3.1. Safety. Pain at the injection site was reported in 49% of vaccinees and 21% of placebo-recipients and headache was reported in 22% of vaccinees and 18% of placebo-recipients. These were the most common adverse experiences. Safety laboratory results were not different between vaccinees and placebo-recipients. There were 40 SAEs (not including HIV infections), but only two (fever, rigors) were deemed study product-related.

As will be described below for efficacy, there was a trend in the increased number of HIV infections in vaccinated subjects who

were Ad5-seropositive at baseline. Some post hoc sub-group analyses reached statistical significance. Thus, although still not fully explained biologically and despite extensive efforts to determine a mechanism, it appears that boosting a pre-existing anti-vector cellular immune response to Ad5 with the vectored-vaccine enhanced HIV infectiousness to vaccinees upon exposure from risk behaviors. More discussion on this adverse safety outcome of the STEP study will be described in the efficacy section below. Similarly, see Section 4.3.7 on the NIH Mini-Summit on Adenovirus-vectored HIV vaccines for more on this safety issue.

4.3.3.2. Immunogenicity. Because of the complexity of collecting, processing, storing, handling, and analyzing appropriate samples for T-cell analyses, it was prospectively planned that a randomly selected 25% of participants would have such analyses performed. Of the 354 participants (including both seropositive and seronegative participants) whose samples were collected for this purpose, 75% of vaccinees produced gamma-interferon ELISpot responses against one or more of the vaccine antigens. ELISpot analyses were performed on samples taken at weeks 8 and 30, subsequent to the 2nd and 3rd doses of vaccine. Table 2 of the Buchbinder article provides the immunogenicity results from the STEP study. The most frequent responses were against the Gag antigen, with 75% of the Ad5-seronegatives responding and 54% of the Ad5-seropositives responding. 84% of the Ad5-seronegatives and 68% of the Ad5-seropositives responded to 1 or more antigens. The strongest responses were seen to the Pol antigen, with GMT of 489 in the Ad5-seronegatives and 245 in the Ad5-seropositives. While Ad5 pre-existing immunity did blunt responses against the vaccine antigens, the majority of subjects still responded with reasonable robustness. Whether this less robust response in the Ad5-seropositives contributed to the apparent enhanced risk of acquisition in this sub-group is unknown, but was among the many factors considered when trying to understand a possible mechanism of action and risk factors for the apparent enhancement seen. Consideration of an immune mechanism of action was also given to the role of immune activation, by the vaccination series, of CD4 T cells, which could be the target of HIV infection, at mucosal surfaces. See the publication on the proceedings of the NIH Mini-Summit described in Section 4.3.7 below.

4.3.3.3. Efficacy. As stated above, the primary efficacy endpoint for the study was focused on the population that was Ad5-seronegative at baseline, i.e., the original trial population. This was, in fact, a co-primary endpoint, of infection rates or viral load (average of two measures taken approximately three months after infection) or both. Efficacy in the entire study population was a secondary endpoint. An assessment of associations between the primary endpoints and prognostic factors was planned as a tertiary endpoint. Prognostic factors included gender, anti-Ad5 titer at baseline, age, ethnicity, HLA type, and circumcision status (in men).

The per-protocol population had 29 infections in men and one in a woman. This woman received the placebo. Further analyses thus focused solely on infections in men. In men, there were 19 infections in the vaccine group and 10 in the placebo group ($p = 0.949$). The mean viral load set-points in these men were 4.6 $\log_{10}$ copies/mcL in the vaccinated group and 4.57 $\log_{10}$ copies/mcL in the placebo group ($p = 0.528$). In the MITT population, there were 45 infections: one in the woman who received the placebo, 24 in vaccinated men and 20 in men who received the placebo ($p = 0.743$). Mean viral load set-points were 4.61 $\log_{10}$ copies/mcL in the vaccinated men and 4.41 $\log_{10}$ copies/mcL in the men who received the placebo ($p = 0.656$). All p-values reported were one-tailed assessing vaccine benefit.

Given the lack of efficacy and the higher rate of infections in the vaccine arm of the study, the tertiary analyses of association became exceedingly important as hypothesis-generating. Doing so permitted the attempt to understand whether there was a sub-population of vaccinated individuals who were at increased risk of HIV acquisition or whether there was simply a lack of efficacy. While these analyses identify only associations and do not test whether the associations are causal, they can shed light on potential modes of action. First, univariate Cox proportional-hazard models were used to quantify treatment effects in the four sub-groups based on the original stratification by baseline Ad5-serostatus. These analyses were shown in Table 4 of the Buchbinder article. The overall hazard ratio for treatment effect was 1.5 (95% CI 0.97–2.3, $p = 0.07$). The interaction of vaccine or placebo and baseline Ad5-serostatus showed a trend ($p = 0.08$). The interaction of vaccine or placebo and circumcision status was statistically significant ($p = 0.01$). The authors urged caution in interpretation however, as subjects were not randomly assigned based on circumcision status. In fact, men who were Ad5-seropositive were much more likely to be uncircumcised than those who were Ad5-seronegative. Race, age, and geography did not show significant interactions. Likewise, behavioral risk factors did not show significant interactions with vaccine or placebo among those who became infected through their risk behaviors. Time-to-event was defined as the time of the original vaccination (or placebo inoculation) to the midpoint between the date of the last visit at which a subject remained HIV-seronegative and the first positive visit (based on first evidence of HIV infection). Further, groups were defined by quadrants of serostatus: Ad5 titers of $\leq$18 (essentially seronegative), 19–200, 201–1000, and >1000. Kaplan–Meier curves were plotted and are shown in Figure 2 of the Buchbinder article. Treatment effects were quantified with estimated Wald-based 95% CI and two-tailed p-values. Finally, multivariate Cox models were used to estimate treatment effects after adjusting for dichotomous confounding factors. Hazard ratios were calculated between those who received vaccine and those who received placebo for the four sub-groups of being Ad5-seronegative and circumcised, Ad5-seronegative and uncircumcised, Ad5-seropositive and circumcised, or Ad5-seropositive and uncircumcised. The results were respectively, 0.7 (95% CI 0.3–1.4), 3.3 (95% CI 0.7–15.8), 1.6 (95% CI 0.7–3.8), and 3.9 (95% CI 1.3–11.9).

One facet that warrants discussion is that while the rates of HIV acquisition in most sub-groups was on the order of about 4%, in the placebo-group of Ad5-seropositives, the rate was almost half this. The contribution of this fact to the analysis cannot be ignored. However, the hazard ratios increased with increasing Ad5 titers, suggesting these findings were not simply an effect of this statistic.

Although the risk of HIV acquisition between vaccinees and placebo recipients did not associate with serostatus for HSV-2, being seropositive for HSV-2 independently associated with the risk of HIV acquisition, regardless of vaccination or placebo receipt. Among 88 men who became infected with HIV through their risk behaviors during the STEP study, 33 vaccinees were HSV-2-seronegative, 18 placebo-recipients were HSV-2-seronegative, 19 vaccinees were HSV-2-seropositive and 17 placebo-recipients were HSV-2-seropositive. Pre-existing HSV-2-seropositivity was associated with increased acquisition of HIV among placebo-recipients ($p = 0.019$) with a HR of 2.2 (95% CI, 1.1–4.2) and among all trial participants ($p = 0.009$) [9].

A “sieve” analysis was also performed for this study and found that breakthrough infections in vaccinees had sequences that were more divergent from the vaccine insert than did the viruses with which the placebo-recipients were infected, suggesting that the immune response did play some role, although it did not result in

clinical benefit (viral load reductions) or protection from HIV acquisition [10].

4.3.3.4. Lessons learned relevant to regulatory evaluation. Despite being possibly the most potent candidate vaccine at the time, which elicited good cellular responses in a significant percentage of participants of the STEP study, the T cell responses alone elicited by this vaccine regimen were insufficient to prevent acquisition of the HIV infection. Further, it was expected that cellular immunity elicited by vaccination would result in decreased viral load among those who did become infected and yet, this was not seen in this study either. Regulators should note that while an applicant should provide immunological justification or a proposed mode or mechanism of action for their vaccine candidate to support conducting clinical trials, for HIV/AIDS vaccines, the field still does not know which types and quantities of immune responses are required for protection. Not only is there no known correlate of protection, but even the immunological mechanism whereby a vaccine might protect, either by preventing acquisition or by modulating disease in those who become infected, is still unknown. When reviewing a CTA, consideration of this lack of knowledge must be taken.

Another relevant lesson learned from the STEP study is that the previously only hypothetical potential for enhanced risk of HIV acquisition is in fact, not simply hypothetical. It is also hypothetical (and this potential risk should and does appear in informed consent forms) that vaccination could paradoxically enhance disease progression in those who subsequently become infected. While this was not seen in the STEP study, only additional follow-up and time will tell. The consequences of these findings and both the demonstrated and hypothetical risks are that trial participants need to be informed of such risks. In reviewing CTAs, the informed consent process and forms should convey the potential risk for a vaccine to paradoxically harm, rather than protect, the participant by enhanced risk of infection through risk behavior or enhanced risk of disease progression in those who become infected.

Potential consideration should be given to use of one-tailed vs. two-tailed p-values in the analysis of efficacy. In the STEP study, one-tailed p-values were considered assessing vaccine benefit, but given the results, it may have been more appropriate to consider two-tailed p-values in which benefit or harm are equally considered. Regulators should have that discussion with clinical trial sponsors and come to an agreement on the appropriate statistical plan before a study is unblinded and the data analyzed. Also, statistical plans for analyzing associations between efficacy endpoints and potential factors that might prognosticate risk of infection should be clear prospectively. This is to permit identifying both potential sub-groups in whom efficacy may be greater and in the event of lack of efficacy, in whom enhanced risk may have been seen. Doing so permits conducting subsequent trials in safer and more appropriate populations and avoiding populations who may be at enhanced risk. This helps to balance risk with potential for benefit in subsequent trial designs.

4.3.4. Phambili

Phambili (pam-bee-lee) was a companion study to the STEP study and was also denoted HVTN 503. The name is Zulu for "Forward!" denoting the urgency to press forward with development of an HIV/AIDS vaccine. The Phambili study was performed in South Africa and enrolled sexually active men and women aged 18–35 in the general population, at risk due to the high prevalence in that country. The vaccine used in Phambili was the same as that used in STEP, with the same doses and regimen. The Phambili study was also intended to be a test-of-concept study (extending the concept to non-clade-matched regions).

The vaccine candidate only encoded internal viral proteins (the capsid protein, Gag, and the polymerase) and a regulatory non-structural protein (Nef) from Clade B. Because of this, it was felt that the degree of conservation among clades for these proteins, which do not experience as much immune pressure to vary as do the Env proteins on the outside of the virus, was sufficient to be useful in the predominately Clade C geography. Furthermore, the study population has a high degree of seropositivity against the vector, Ad5, with many seropositives having higher titers than in geographies where there is lower seropositivity in the population. This study was also a Phase 2b test-of-concept efficacy study, like STEP. It was conducted by the HVTN with support from Merck. This study represented a very difficult challenge for the vaccine, as opposed to the original population in the STEP study, in which individuals did not have pre-existing immunity to the vector and the vaccine candidate was clade-matched to the predominant circulating HIV strains. In the case of Phambili, it was a cross-clade "challenge" and individuals could theoretically have experienced blunting of the immune response to the vaccine due to pre-existing anti-vector immunity. However, this study population represents one of the higher risk populations globally, reflecting a more "real-world" situation for which the vaccine was tested. Phambili was initiated before the results of the STEP study were known, and as the name of the study implies, due to the urgency to press forward. Enrollment was performed between January and September 2007 and was halted due to the interim analysis of the STEP study revealing potential enhancement of HIV acquisition risk in Ad5-seropositives in STEP. Thus, only 801 of the originally planned 3000 individuals were enrolled. This two-arm study randomized 1:1 vaccine to placebo.

The study was designed to be event-driven with the expectation of observing 120 HIV infections in 3000 participants, giving 80% power to detect: efficacy against HIV acquisition of $\geq$45%, $\geq$0.75 log difference in viral load at about two months and three months (average) in those who did become infected, or both. Because of the early halt to the study, the planned per protocol analysis was rendered essentially meaningless (under-powered due to sample size) and so a modified intent-to-treat analysis was used instead. The MITT analysis included everyone vaccinated (vaccine or placebo) except those who were later determined to already be HIV-infected at enrollment.

Of those enrolled, 400 received vaccine and 401 received placebo. Respectively (vaccine, placebo), 112 and 104 received only their first injection, 259 and 270 received their first two injections and only 29 and 27 received all three planned injections prior to the early halting of the study. The study enrolled 441 men and 360 women. Reported in publication, there were 62 infections, 42 of which were in women. More recent data from the study, which was halted for vaccinations, but permitted continued follow-up of unblinded participants for safety, including HIV acquisition, has revealed several more infections; 61 in women and 39 in men. Follow-up for infections and safety continued by re-enrolling participants in a "roll-over" study.

The scientific controversies to this study were in regards to its timing relative to the STEP study, i.e., before the results of the STEP study, which enrolled the "best-case" population, were known and to using a clade B vaccine candidate in a population at risk of clade C infections, as discussed above.

Some information in this section and subsequent sub-sections has been taken from the publications of the efficacy study results by Gray et al. [11,12]. Information has been paraphrased or sometimes directly quoted.

4.3.4.1. Safety. Table 2 in the Gray et al. (2011) [11] publication outline safety data from the Phambili study. Briefly, the most

common adverse events were reactogenicity. Local pain or tenderness occurred in 62% of vaccinees and 33% of placebo-recipients. Any systemic reactogenicity occurred in 66% of vaccinees and 55% of placebo-recipients, with headache (46% vs. 37%) and malaise or fatigue (39% vs. 29%) being the most frequent of these. Reactogenicities that reached statistical significance in differences between the two arms of the study were the local pain and tenderness ($p < 0.0001$), headache ($p = 0.01$), malaise or fatigue ($p = 0.06$), myalgia ($p < 0.0001$), and arthralgia ($p = 0.03$). Severities were not reported in the publication. Among the non-reactogenicity category of adverse events, none that were experienced by participants reached statistical significance in rates between the two arms of the study.

Pregnancies did occur during the study despite counseling to use adequate birth control to prevent them. The rates did not differ between study groups. Sexually transmitted infections, other than HIV infection, were also reported in participants, occurring more frequently in women (11.1 vs. 6.7 per 100 person years, $p = 0.007$), but occurring equivalently between study arms (7.5 vs. 9.8 per 100 person years) and before and after the trial was unblinded (9.9 vs. 9.6 and 7.7 vs. 9.9 per 100 person years).

Unlike the STEP study, statistical trends towards enhanced acquisition of HIV infection in vaccinees who were Ad5-seropositive at baseline or men who were uncircumcised were not observed. But, it should be noted that the study was unpowered significantly by virtue of being halted early for enrollment and vaccination, when only 27% of the planned study population was enrolled. So, this adverse safety signal seen in STEP was not substantiated in Phambili. However, there were more HIV infections in the vaccinated group than the placebo group, as discussed below, suggesting the lack of efficacy of this vaccine candidate.

4.3.4.2. Immunogenicity. Immunogenicity was assessed primarily at the point four weeks after the 2nd injection (for those who received more than one). Most vaccinees developed gamma-interferon-secreting T cells against Clade B peptides and more than three quarters against Clade C peptides. Table 3 in the Gray et al. (2011) [11] publication outlines these data. Highest frequency of responses was to the clade B Gag antigen with 89% of Ad5-seronegatives and 77% of Ad5-seropositives responding. Substantial proportions responded to the other two clade B antigens as well and to more than one antigen, with more than half responding to all three clade B antigens. In the case of clade C antigens, the highest frequency of responses was seen to the Pol antigen, with 83% of Ad5-seronegatives and 67% of Ad5-seropositives responding. More than half of vaccinees responded to at least two of the three clade C antigens. Ad5-seronegatives had consistently higher response rates than Ad5-seropositives, reaching statistical significance for some antigens or for each clade overall. Magnitudes of responses were not reported in the publication. Given the relatively high frequency of responses to Clade C antigens by vaccination with the Clade B vaccine, the rationale to press forward appears sound.

4.3.4.3. Efficacy. Although the study was halted early, there were infections due to risk behavior, which occurred on study, as expected in an efficacy study despite risk reduction counseling. As mentioned above, a MITT analysis was performed. Overall, 34 infections occurred in vaccinated subjects and 28 in placebo-recipients. In women, 22 vaccinees and 20 placebo-recipients became infected. In men, infections occurred in 12 vaccinees and eight placebo-recipients. The hazard ratio for infections in women to men was 1.25 (0.76–2.05 95% CI). Time to infection was also analyzed. The incidence rate for vaccinees vs. placebo-recipients was 4.54 vs. 3.70 per 100 person years. Incidence rates were high in women in both groups, 6.79 in women in the vaccinated group and 5.86 in women in the placebo group.

Predictors of infection identified in a multivariate Cox proportional hazard model analysis were gender, age, seropositivity to herpes simplex type 2 (HSV2) and the interaction between gender and HSV2 (increased risk in men, but not women). In fact, when the analysis was restricted to women, no predictors of infection were noted. Parameters that did not significantly predict infection were baseline serostatus or titer to Ad5 (using <18 or >200), number of inoculations received, or behavioral risk factors. Further, age and baseline titer to Ad5 did not differ by gender among those who became infected on study. In men, neither baseline Ad5 titer nor circumcision status had significant association with HIV infection.

Viral load setpoint, as an average of plasma RNA levels at about two months and about three months post-infection, were obtained for 61 of those infected on study. There was no difference in distribution of viral load setpoint between study arms, stratified by gender. Women tended to have lower viral load setpoints than men, but this difference was not significant ($p = 0.15$). Also, women who were vaccinated tended to have lower viral load setpoints than women who received placebo, but this difference was also not significant ($p = 0.16$). Participants who had baseline Ad5 titers <18 (Ad5 seronegative) tended to have higher viral load setpoints than those who had titers >18 (Ad5 seropositive), but again, this was not significant ($p = 0.12$).

The co-primary endpoints, with statistical adjustment ($p = 0.39$), were not significantly different between study groups. Therefore, the vaccine candidate was not demonstrated to have efficacy.

After long-term (42 months) follow-up, 63 (35 women, 28 men) vaccinees and 37 (26 women and 11 men) placebo-recipients had become infected through risk behavior. Figure 2 in the Gray et al. (2014) [12] publication shows how the Kaplan–Meier curves appear to diverge after time on study, with infections accruing more rapidly among vaccinees. Whether this is the effect of differential drop-out after unblinding (7.7% annualized rate for vaccinees and 8.8% annualized rate for placebo-recipients) or some other confounder is not clear, although analyses were performed suggesting this was not an artifact of differential drop-out rates in the two study arms.

4.3.4.4. Lessons learned relevant to regulatory evaluation. As with all trials, many lessons are learned operationally and in terms of study design, with regards to epidemiology in the study population, about social harms and many other facets. Phambili was no different in this regard.

One scientific lesson learned from the combination of the STEP study and Phambili was that a vaccine candidate that solely elicits T cells is unlikely to prevent HIV acquisition and may not impact post-infection control of viral load predicted by animal models. The Merck Ad5-vectored HIV vaccine candidate, expressing clade B Gag, Pol, and Nef, elicited strong T cell responses in a majority of those vaccinated, even if individuals were Ad5 seropositive (i.e., had pre-existing anti-vector immunity). However, this candidate vaccine failed to demonstrate efficacy in two separate studies separated by geography, behavioral risk factors for infection, incidence rates, genders at greatest risk of infection and other relevant factors. As a consequence, subsequent vaccine development by the field will primarily be aimed at eliciting a combination of antibodies and T cells. It is still felt, in the scientific community, that T cells are essential to a successful HIV/AIDS vaccine, if for no other reason than CD4+ T cells are important for antibody elicitation and function. In fact, it is likely that CD8+ T cells will also be important, but not sufficient, in a successful vaccine. Thus, as regulators review CTAs, the proposed immunological mode or mechanism of action of

a prophylactic vaccine candidate or regimen should be made clear to them and evidence provided for immune responses that may include, but not be limited to, cellular immunity.

One surprising lesson learned from the more recently published data [12], is that there may be value to not unblind a efficacy study that has had enrollment and vaccinations halted due to the futility of the study to demonstrate efficacy, but which continues in follow-up for safety, including acquisition of HIV infections. By having unblinded the Phambili study when it was halted, it is impossible to know whether there has been bias introduced into the rate of acquisition of HIV infections between individuals who know they received vaccine and those who know they received placebo. There may even be differential retention between the study groups because of participants' perception, which may have confounded the more recent results. Statistical analyses have been performed to illuminate these potential problems, but it is impossible to know, because the study was unblinded when enrollment and vaccinations were halted. Unfortunately, this lesson was learned a little too late for the HVTN 505 study, which is described below. For future efficacy studies, due consideration should be given to balancing the ethics and the right of participants to know in which study arm they were enrolled against the scientific and societal value of continued follow-up in the study in a blinded manner, when a study is halted early for futility. This is especially the case when there appears to be a trend towards enhanced acquisition in the vaccinated arm – a safety issue that warrants follow-up and further scientific study and analysis in order to determine how to prevent such safety issues in future efficacy trials with related, or even unrelated, vaccine candidates. Understanding the mechanism of action of enhancement, if it occurs, is as important as understanding correlates of protection for a successful vaccine. But ethical considerations will take precedence, as they always should.

4.3.5. RV144

Originally conceived and funded by the U.S. Military HIV Research Program (USMHRP) and the Ministry of Public Health of Thailand and ultimately also funded by the Division of AIDS at the U.S. NIH, the RV144 study was conducted in Thailand. RV144 enrolled from a general population of healthy adults aged 18–30 at primarily heterosexual risk but including MSM, commercial sex workers (CSW) and IDU/PWID based on self-reported risk behaviors. This study combined the bivalent B/E gp120 vaccine used in the VAX003 study from Global Solutions for Infection Diseases (GSID, http://www.gsid.org/, accessed 8/19/2013, USA), who were granted rights to the product from VaxGen, with an ALVAC vector prime in a regimen that delivered ALVAC at all four doses and gp120 at the final two doses, at 0, 4, 12, and 24 weeks. The ALVAC vector, vCP1521, was based on the ALVAC from Virogenetics. It expressed Gag and Pro from clade B (LAI strain) and gp120 from clade CRF_01 (A/E) strain 92Th023, linked to the transmembrane anchoring domain of gp41 (LAI strain) and was made by sanofi pasteur. This study was conducted as a complex partnership of multi-national public and private interests.

Participants were randomized 1:1 vaccine regimen vs. placebo. The study enrolled 16,402 participants. Originally, the primary endpoint was the acquisition of HIV infection, but after controversy was raised in the scientific community by the conduct of the study, a co-primary endpoint of viral load was added. The viral load endpoint was an average of three measures taken within six weeks of serodiagnosis. The immunogenicity analyses will be further described in the sections below on immunogenicity and on the correlates analysis.

Although it may have been conceived as such originally, this Phase 3 study was not designed to support licensure as a sole trial. The sample size selected in the target population studied gave only sufficient power to assess efficacy with a lower bound of the confidence interval to exceed zero, and not 30%, as had been recommended to the U.S. Food and Drug Administration (FDA), who reviewed and approved the study, by their committee of external advisors. So, while the sample size was quite large, the study still risked being underpowered to provide compelling evidence of efficacy if the point estimate of efficacy was below 50%, as it turned out to be. Nonetheless, the study served as a test-of-concept or pilot efficacy study. The data from this study could be considered in conjugation with another trial of the same or related regimen to support licensure if convincing and concordant results were obtained, but such a study has not been completed or conducted at this time. Plans to do so are described in the companion review.

The statistical analyses of the primary endpoint warrant some discussion, because some of the controversy that arose over the results of the study was based on the difference in achieving statistical “significance” between the per-protocol and the MITT analyses. A Cox proportional-hazards method was used. The planned per-protocol analysis was to include all subjects who received each of their study treatments within the required time-window, acquired HIV infection after completion of the entire series, and were not excluded for other reasons. The ITT analysis included all subjects randomized to the study. The MITT analysis included all randomized subjects who received their first inoculation and who were not subsequently determined to be in the window period of HIV infection (i.e., screen seronegative at baseline, so qualified for the study, but later were found to be HIV plasma RNA positive at first inoculation).

A futility analysis was also conducted at planned interim analyses by an independent DSMB, which met every six to 12 months (8 times during the study). The futility statistic would have led to halting the study had the conditional power to detect a difference in VE between groups fell to less than 10%. Reported p-values were two-tailed, considered significant at the 0.05 level and were not adjusted for multiple testing.

Controversy over the design and conduct of this study began after the study was open and enrolling subjects. There were objections within the scientific community that the candidate regimen was not sufficiently potent to warrant the expense of conducting such a large efficacy study, particularly one that would not in itself support licensure. At that point in time, T cell vaccines were thought by many scientists to be the best hope, because broad neutralizing antibodies were yet to be attained by any candidate or regimen. Since this regimen did not elicit potent or frequent T cell responses, several scientists objected to moving forward with the regimen. As will be noted among the lessons learned, without a predictive animal model, despite what may be the prevailing scientific opinion, it is impossible to know what vaccine candidates or regimens will elicit protective efficacy against HIV infection and AIDS without conducting an efficacy trial in humans. While some scientists may have the hubris to believe they know what will or will not work without having done the experiment, regulators must remain agnostic. Human efficacy trials must be conducted in order to know if a candidate HIV/AIDS vaccine will have efficacy. Although at the time, T cell vaccines were favored, as subsequently learned from the failure to see efficacy in the STEP and Phambili studies, this favored hypothesis has proven faulty. While many in the HIV/AIDS vaccine field did not believe that efficacy would be demonstrated in this study, in fact, it is the only clinical trial so far that has demonstrated any efficacy, albeit quite modest. As a consequence of these objections raised after the study was already enrolling subjects, the primary endpoint was modified to include a co-primary endpoint of viral load and CD4 counts among those who would become infected on study due to their risk behavior.

Controversy also arose over the results of the study, when there was discordance in the statistical significance for VE based on the per-protocol analysis vs. the MITT analysis. In part, this can be understood on the basis of imperfect adherence to the complete vaccination series, which resulted in a loss of nearly 4000 of the 16,000 counted in the per-protocol analysis. Since the study sample size was selected to rule-out zero as the lower-bound of the confidence-interval, the loss of nearly 25% of participants from the sample size analyzed had a significant impact on the power of the per-protocol analysis to achieve significance and rule-out zero in the confidence-interval. In contrast, only those individuals found subsequently to actually have already been HIV-infected at baseline ($n = 7$; i.e., nearly all 16,000 were included in the analysis) were eliminated in the MITT analysis, giving it substantially more power to detect differences between incident infections in the study arms. However, for those unfamiliar with the nuances of clinical trial statistical analyses, the fact that the various analyses were not concordant in their statistical significance caused many to doubt the results. These doubters considered the statistically significant results to be a statistical "fluke." In fact, the reason the data are analyzed in these various ways (PP, ITT, MITT) is exactly to divine whether a particular analysis is a fluke or whether the analyses do gain concordance and thus, enhance confidence in the results. In addition, the impact of waning immunity on the differential rates of infection between study arms raised concerns. The number of infections that occurred during the time of peak immunity in the vaccine arm was insufficient to have power to detect a statistical difference, although there was a clear difference. But by the time sufficient events had occurred to have the necessary power, the rates of infections between the two groups were aligning, because the impact of the vaccine regimen was waning. However, the findings of a correlates analysis, described below, have allayed the concerns among at least some of these doubters.

Some information in this section and subsequent sub-sections has been taken from the publication of the efficacy study results by Rerks-Ngarm et al. [13]. Information has been paraphrased or sometimes directly quoted. Additional information on the outcomes of this study in regards to an independent statistical evaluation (given the controversy of the analyses of the study) and behavioral risks, respectively, can be found in the literature, but are not summarized here [14,15].

4.3.5.1. Safety. Local and systemic reactogenicity seen was mostly mild to moderate and occurred at similar frequencies and severities in both study arms. Most reactions resolved within three days post-vaccination. The number of deaths and the frequency and severity of SAEs were also similar between the two study arms. More details were provided in Supplemental Figure 1 and Supplemental Tables 1a, 1b, and 1c in the Rerks-Ngarm article (Rerks-Ngarm, 2009 [13]). For example, the rates of AEs and SAEs in the 30-days post-any-vaccination were respectively, 32.4% and 1.5% in the vaccine arm and 33.4% and 1.8% in the placebo arm. The majority of SAEs were injuries and the authors have related publicly that motorbike accidents are an all-too-common occurrence in Thailand. Thus, there was no indication of a safety signal related to the vaccine regimen. More detailed safety data were published separately [16].

4.3.5.2. Immunogenicity. In reality, as was the subject of objection to the trial, the vaccine regimen was poorly immunogenic in regards to T cells, although antibody responses were prevalent. Nearly all vaccinees developed anti-gp120 and anti-Gag antibodies. GMT to MN gp120 were 13,207 and to A244 gp120 were 14,588. Anti-p24 antibodies were of lower frequency with GMT of only 138. Lymphoproliferation was also measured and was significantly higher in vaccinees. Only 19.7% of vaccinees developed and maintained cellular responses at six months post-last-vaccination as measured by gamma-interferon ELISpot to either Env or Gag antigen. These analyses were the planned immunogenicity measures in the protocol. However, after efficacy was seen, a correlates analysis was planned and executed and will be described below. This trial is the subject of a recent review [17].

4.3.5.3. Efficacy. The analysis for VE, i.e., prevention of HIV acquisition, was performed in three ways, as already alluded to above. The per-protocol analysis included all subjects who received the complete vaccination series within the defined time windows and who were not excluded for other reasons, including acquisition of HIV before completion of the vaccination series. The ITT analysis included all subjects randomized into the study. The MITT analysis included all randomized subjects who received their first vaccination (at least) and who were not subsequently found to be already HIV-positive at baseline (positive for HIV RNA in the plasma, although screened seronegative), i.e., only seven subjects were excluded from the MITT analysis. Each of these analyses was conducted by a Cox proportional-hazards method.

The ITT analysis included 132 infections − 56 in the vaccine arm and 76 in the placebo arm. VE was estimated at 26.4% with a 95% CI of −4 to 47.9%, $p = 0.08$. The per-protocol analysis included 86 events − 36 in the vaccine arm and 50 in the placebo arm. VE was estimated at 26.2% with a 95% CI of −13.3 to 51.9%, $p = 0.16$. Finally, the MITT analysis included all but seven infections that were present at baseline for a total of 125 infections − 51 in the vaccine arm and 74 in the placebo arm. VE was estimated at 31.2% with a 95% CI of 1.1–51.2%, $p = 0.04$, achieving statistical significance. Other analyses of the MITT population gave statistically significant results, as follows: Barnard's test ($p = 0.04$), log-rank test ($p = 0.04$), Wilcoxon test ($p = 0.03$), modification of time to seroconversion endpoint ($p = 0.04$), exclusion of hospital-diagnosed infection ($p = 0.05$), and analysis of interval-censored data ($p = 0.04$).

Subjects' viral loads were assessed at time of diagnosis, three weeks, and six weeks subsequently, and averaged. Mean viral load and CD4 counts in those who became infected on study did not differ between groups. Mean viral loads were 4.36 $\log_{10}$ copies/mcL in the vaccine arm and 4.21 $\log_{10}$ copies/mcL in the placebo arm in the ITT population ($p = 0.09$), 4.24 $\log_{10}$ copies/mcL in the vaccine arm and 4.19 $\log_{10}$ copies/mcL in the placebo arm in the per-protocol population ($p = 0.47$), and 4.30 $\log_{10}$ copies/mcL in the vaccine arm and 4.20 $\log_{10}$ copies/mcL in the placebo arm in the MITT population ($p = 0.24$). Likewise, mean CD4 counts between the vaccine and placebo arms, respectively, were 541 cells/mcL and 568 cells/mcL in the ITT population ($p = 0.47$), 572 cells/mcL and 532 cells/mcL in the per-protocol population ($p = 0.72$), and 555 cells/mcL and 568 cells/mcL in the MITT population ($p = 0.76$).

The Kaplan–Meier curves showing accumulation of HIV acquisition endpoints over time on the study, published in Figure 2 in the Rerks-Ngarm article, are instructive. They show that the rates of infections in the two study arms were similar before completion of the full vaccination series, but in the six months to year following complete vaccination, the arms diverge. In the MITT analysis that included infections occurring before the vaccine series was complete, divergence begins to be seen even earlier (after two or three vaccinations). Subsequently, however, the study arms become parallel in their slope as new infections accumulate in both study arms at approximately the same rate. These figures lead one to surmise that the efficacy that was seen waned, as immune responses likely waned. Although the final statistically significant result (MITT) was 31.2% efficacy seen at three years post-last-vaccination, at six and 12 months post-last-vaccination, the point estimate of efficacy was closer to 60%. It is noted however, there

were too few events at those points in the study to see a statistical difference between the study arms.

4.3.5.4. Correlates analysis. Subsequent to the completion of the RV144 study, a correlates analysis was planned and implemented. A large number of analyses were performed, but six were prospectively identified as primary analyses. One of these parameters, anti-V1V2 IgG antibodies, correlated inversely with risk of HIV acquisition in vaccinees, whereas another, IgA antibodies, correlated directly with risk of HIV acquisition among vaccinees. Identified correlates of risk (those with inverse correlations) could be evaluated in future efficacy trials as potential correlates of protection. It should be borne in mind that a correlate of protection for one vaccine candidate or regimen may not be the same for a different candidate or regimen that is expected to have a different mechanism or mode of action. This immune correlates analysis is published, as is a review of the lessons learned [17,18].

4.3.5.5. Lessons learned relevant to regulatory evaluation. As stated, despite wide-spread scientific controversy about the likelihood that this candidate regimen would demonstrate efficacy, this was the only efficacy study performed so far in which modest efficacy was seen. This fact emphasizes that we must remain humble and agnostic about outcomes of scientific studies until the well-controlled, well-designed studies are performed and the results become known. Regulators should take note of this uncertainty and while it is requisite to provide scientific justification to support the conduct of any clinical trial, the lack of a predictive animal model or natural survivors in the HIV/AIDS field precludes reliance on such data to justify going forward with any particular vaccine candidate or regimen. Immunogenicity data from human clinical trials and a plausible proposed mechanism of action are necessary to proceed to efficacy studies, but which candidates or regimens should advance and which do not warrant the effort, resources, time and expense remains controversial within the HIV/AIDS scientific community. In fact, to date, the most potent of candidate regimens – arguably the Merck Ad5 vector (described above) and the VRC's DNA prime-Ad5 boost (described below) – have failed to achieve efficacy in prevention of acquisition or in control of viral load in those who become infected through their own risk behavior after vaccination. In fact, the Merck vector (solely eliciting T cells) may have been associated with enhanced risk of HIV infection, while the less potent candidate regimen studied in RV144, did achieve some modest degree of efficacy in terms of prevention of HIV acquisition. Clearly, we still have a lot to learn about what types and amounts of immune responses are needed for efficacy.

One major consideration that was taken by the Government of Thailand was the impact of efficacy outcomes in VAX003 on the conduct of RV144, once the outcome of VAX004 became known, since one component of the regimen in RV144 was the vaccine tested in VAX003. A consultation was held by the WHO-UNAIDS HIV Vaccine Initiative to advise the Ministry of Public Health of Thailand in this regard. Consideration was given to different potential outcomes, including high efficacy, modest efficacy, and low or no efficacy. (At that time, the potential for enhancement had not been seen and was not considered.) RV144 showed that even if a component of a regimen is insufficient on its own to elicit protective efficacy that does not mean a priori that it cannot contribute as an element in a successful regimen. While it has not been definitively established that the efficacy seen in RV144 was not elicited solely by the ALVAC vector, because it was not tested alone, the correlates analysis suggests strongly that there was contribution from the protein boost. Thus, the combination of the prime-boost regimen enhanced whatever immune responses resulted in protective efficacy, which were not elicited by the protein alone. Whether this was a qualitative or quantitative difference or both is as yet unknown.

A lesson learned relevant to regulatory evaluation is the consideration of which analysis is the most relevant for vaccine efficacy. Generally, one would consider that the per-protocol analysis, which is selected to analyze the best-case-scenario for a vaccine, would be the most relevant. Intent-to-treat analyses are often relevant in the case of drug trials because of differential adherence to a drug regimen. Vaccine regimens, in contrast, are short (generally three to five doses, but given over months) and thus, considerably easier for adherence. Also, they are given by clinicians rather than the trial subject themselves, so adherence can be definitively established. Given the window period for HIV infection and the months between the first dose of the vaccine and the last, arguably the most relevant analysis is the modified-intent-to-treat analysis. The MITT includes every subject who received at least their first inoculation (vaccine or placebo) and who subsequently is shown to be HIV-uninfected at baseline (some individuals who are seronegative at baseline may actually be infected and in the "window period", and identified later as being infected at baseline). Although the MITT population does not represent the "best-case-scenario" in which the full vaccination series is given and time elapsed to permit an adequate immune response to develop, the MITT analysis reflects a real-world scenario of protecting uninfected individuals by vaccination, since they may become infected anytime during or after the course of the vaccination series. In the case of RV144, only the MITT analysis achieved statistical significance. In part, the per-protocol analysis did not achieve significance, because power was lost by virtue of problems in adherence to the full vaccination series (mostly due to "falling out" and missing the prospectively defined window of opportunity for vaccination even though the vaccinations were given, just not on time). So fewer individuals (12,542 out of the 16,402) were included in the per-protocol analysis – in contrast to the MITT analysis, which included 16,395 of the 16,402 enrolled. Only 86 infections were included in the per-protocol analysis, while 125 infections were included in the MITT, enhancing its power to detect differences. Clarity on the analysis plan should be provided by the applicant for regulatory evaluation. The statistical analysis plan should be provided prospectively for regulatory evaluation. Regulators should play particular heed to the MITT analysis in vaccine studies, because of the timing of vaccine dosing and exposure to the disease organism. Ideally, all analyses will be concordant, enhancing confidence in the results. When discordant results are seen, attention should be paid to which analysis was significant and why the analyses may have been discordant.

Furthermore, regulators should take note of the issues that would impact credibility of the trial results and seek clarity in how a study sponsor plans to power and analyze an efficacy study, to avoid a loss of confidence in the study outcomes. Credibility of the results is essential to regulatory decision-making. It is for this reason, among others, that FDA's external advisors recommended licensure decisions only be made on an efficacy study in which the lower bound of the confidence-interval exceeded zero by a good margin (e.g., 25–30%). This requires either a point estimate of efficacy be reasonably high ($\geq$50%) or the trial be designed to have sufficient precision to have a tight confidence-interval around the point estimate or both.

The issue of waning immunity is another issue on which regulators should be concerned when reviewing CTAs. The study design should address this and might do so in multiple different ways. One option might be to continue to boost subjects every six months or yearly during the study, in a multi-year follow-up study. Of course, this approach would have implications for how the vaccine would need to be used, once licensed. Another option would be to conduct

the study in a population at significantly higher risk than was seen in RV144. In that manner, more events would occur in a shorter time-frame potentially obviating the need for a three-year follow-up. However, such a population might represent a more stringent challenge for the vaccine regimen and may not be generalizable to the populations in which any particular country might wish to deploy a successful vaccine. As a final option, the sample size of the study might be increased in order to have sufficient events in a shorter time-frame of follow-up, but this raises several logistical and operational challenges (and potentially ethical ones as well, as more subjects could be put at risk before the vaccine is revealed as beneficial or not) and may not be feasible. Furthermore, the time to enroll such a large sample would likely take multiple years itself, further complicating the study and the analyses, as bias could be introduced over time due to a changing landscape in both standard-of-prevention and standard-of-care (including treatment-as-prevention and effects on viral load as a study endpoint).

The correlates analysis from RV144 is instructive in many ways. First, the analysis would have been greatly facilitated had more of the "right" samples been taken. Samples taken in the right frequency and time-frame post-vaccination and post-infection would have greatly enhanced the ability to discern meaningful differences between vaccinees who became infected and those who may have been protected. The "right" samples in terms of serum, plasma, or cells, in terms of blood or mucosal specimens and so forth, must also be considered. All of this and the potential to enhance the science and knowledge gained from a hopefully successful efficacy study needs to be weighed against the logistics and operational costs (resources, time, and expense) of additional subject visits; objectionable and potentially more risky, more invasive sampling procedures; additional processing and storage of samples; increased complexity of study design and analysis; potentially increased difficulty in recruitment and retention; and the impossibility of knowing a priori who will be the ones that become infected and who will be protected (precluding focus on obtaining more specimens from those).

More lessons have been learned from the correlates analysis. The importance of prospectively prioritizing the analyses cannot be minimized when considering reducing the power to define meaningful differences. Recognition should be taken and plans made accordingly to account for new techniques that will become available, standardized, validated and meaningful between the time the study is planned and initiated and when the analyses (if batched and not performed "real-time") are performed at the end of the study. Also relevant to regulatory considerations is the difference between a pilot efficacy study in which correlates of protection or correlates of risk for infection, as identified in RV144, are hypothesis-generating, vs. the hypothesis-testing of correlates of protection that ideally would be accomplished in a pivotal efficacy study. Given the state of the HIV/AIDS vaccine field however, even pivotal efficacy studies may be performed with correlates analyses that are hypothesis-generating, as correlates of protection have yet to be identified and validated. Further, it is feasible for vaccines with differing mechanisms of actions (types and amounts of different kinds of immune responses, such as antibodies or cellular or systemic or mucosal, different functions of antibodies, different functions of cells, etc.) that there could be different correlates of protection. So, even if the correlates of risk identified in RV144 are shown to be correlates of protection in future efficacy studies of poxvirus primes with protein boosts, another candidate regimen (e.g., DNA-prime, poxvirus-boost) may have different correlates of protection. To understand this better, it might be helpful to think about the differences between the oral poliovirus or Sabin vaccine and the inactivated poliovirus or Salk vaccine. While both elicit systemic antibody responses that are sero-protective in the individual vaccinee, it is believed that some of the mode of action of the Sabin vaccine is via establishing mucosal immunity in the gut, which is the portal of entry for polioviruses. Limiting infection in the gut not only protects the vaccinated individual but also reduces the virus shed into the environment, interrupting transmission. The Salk vaccine is not thought to have this transmission-blocking effect.

4.3.6. HVTN 505

The HVTN 505 study was originally planned as PAVE 100, a multi-network, multi-national study of the Vaccine Research Center's (VRC, NIAID, USA) regimen using the following combination of vaccines in a prime-boost approach. The priming doses, a series of three given one month apart and delivered by Biojector® 2000 needle-less injection system, consist of a mixture of six DNA plasmids expressing Gag, Pol, Nef, and Env from clade B, and two additional Env proteins from clade A and C. The dose of this mixture given was 4 mg total at each priming inoculation time. The single boost dose given at month six, after a four-month rest from the priming series, consists of a mixture of four Ad5-vectors expressing Env from clades A, B, and C and a Gag-Pol fusion protein from clade B. The dose of Ad5 vector given was 1×10^{10} particle units (PU) total.

The PAVE 100 study was due to open for enrollment the week after the STEP study results were announced and that study was halted for futility. Because the STEP study used an Ad5 vector and it appeared that there may have been enhanced risk of acquisition of HIV infection in those vaccinated who were Ad5-seropositive at baseline, controversy was raised whether there was equipoise to conduct PAVE 100. PAVE 100 would have enrolled, among others, Ad5-seropositive individuals and men who were uncircumcised. As a consequence of this controversy and turn of events, PAVE 100 was abandoned, additional supportive animal data were gathered to assess the risk of enhanced acquisition, as well as protective efficacy, with the animal model analogs of the VRC regimen, and the clinical study was redesigned.

HVTN 505 was, consequently, conducted solely in the U.S. in circumcised men who were Ad5-seronegative MSM or transgender (male-to-female). It was designed as a test-of-concept Phase 2b study. It was originally designed to enroll 1350 participants and focus on a viral load endpoint as primary efficacy indicator, but with additional supportive animal data, the study was increased to enroll 2200 participants with a co-primary endpoint of acquisition of HIV infection and viral load in those who became infected on study from risk behavior. The results of PrEP studies were announced while HVTN 505 was enrolling. So, the final accrual goal was raised to 2,500, to account for potentially decreased acquisition endpoints from PrEP use. PrEP use was permitted on study, because PrEP was viewed as likely to become standard-of-prevention in the U.S. and could not ethically be discouraged. In the end, 2504 volunteers were enrolled before an interim analysis for efficacy determined that the study should halt vaccinations due to the futility to be able to demonstrate either co-primary endpoint. The study was unblinded as soon as possible thereafter so that participants would know which study treatment they received. Counseling on risk reduction continued to be provided and the study was continued in follow-up for safety, including acquisition of HIV infection.

Information in this section is quoted directly or paraphrased from Hammer et al. [19].

4.3.6.1. Safety. In general, DNA plasmid vaccines have been found to be quite safe and well-tolerated. In particular, the VRC's six-plasmid candidate prime vaccine, resulted predominately in local

reactogenicity, with less frequent mild systemic reactions. Urticaria has been noted infrequently in studies of the VRC's DNA prime. One point that bears remark is that the Biojector®, when used to deliver the DNA plasmid vaccine, sometimes results in a small lesion at the site of injection. These lesions frequently go unnoticed by the subject, resolve, and have not resulted in any adverse consequences to date. The Ad5 boost results in more systemic reactions than the DNA prime, with headache, malaise, and myalgia being the most common. In both cases (DNA prime, Ad5 boost), reactions were self-limiting and resolved generally within 24–48 h. In HVTN 505, vaccinees had significantly higher rates of reactogenicity than did placebo-recipients. Most reactions were mild to moderate. Non-fatal, non-reactogenicity adverse experiences were balanced between vaccinees and placebo-recipients. Only one severe AE was judged to be related to vaccination, a severe viral syndrome (it should be noted that the Ad5 vector is not replication-competent in humans or in cells other than those that have been engineered to complement the defects in both the Ad5 E1 and E4 ORF6 genes). Six subjects, placebo-recipients, died on the study.

4.3.6.2. Immunogenicity. The VRC regimen elicits robust antibody and T cell responses. In HVTN 505, 61.5% of vaccinees developed vaccine-specific CD4 T cells and 64.1% of vaccinees developed vaccine-specific CD8 T cells, as determined by Intracellular Cytokine Staining (ICS). Predominant targets of these T cells were Gag (48.7%) and Env (38.5%) for CD4 T cells (responses on order of 0.1% of total T cells) and Env (56.4%) for CD8 T cells (0.2%). 100% of vaccinees developed IgG antibodies to HIV Env of the vaccine strains (A, B, and C clades), to a Group M consensus Env (gp140, a truncated gp160), and to the gp41 component of Env. 48% of vaccinees developed IgG antibodies to the gp120 component of Env. IgG responses to V1V2 domains of Env were low (18% to the antigen used in the correlates analysis of RV144 and 20% to a VRC Clade A strain matched to the vaccine). Serum IgA responses were 43%. Neutralizing antibodies were also low (2.5–27.5%) and present only to Tier 1 virus strains (the easier-to-neutralize viruses, see the companion review for more information about this categorization scheme).

4.3.6.3. Efficacy. The HVTN 505 study was halted early at the recommendation of the DSMB, in April 2013. The study was fully enrolled and most participants were fully vaccinated. The primary endpoint was infections occurring between week 28 on study (after the entire vaccination series) and month 24. At the time of the DSMB review, there were 27 vaccinees and 21 placebo-recipients who had become HIV infected in this period of time. Vaccine efficacy was estimated at −25% (95% CI, −121.2 to 29.3, $p = 0.44$). Overall, there were 41 vaccinees and 31 placebo-recipients who had become HIV infected on study inclusive of the primary endpoint window and all other timepoints on study ($p = 0.028$). Thus, the DSMB recommended that efficacy could not be demonstrated and the study should be halted for futility. Subsequent data up until September 2013, as presented at the NIH Mini-Summit, discussed in Section 4.3.7 below, revealed additional infections that evened out the rates of infection between the two arms of the study. There were 43 vaccinees and 39 placebo-recipients infected during the study by the time of that analysis (presented at the Mini-Summit).

Viral setpoints were, on average, 4.46 and 4.47 $\log_{10}$ HIV RNA copies/mL, respectively, in the vaccine and placebo groups, in individuals who had become infected on study. Thus, the vaccine regimen lacked both the ability to prevent acquisition of HIV infection or to modulate viral load in vaccinees who became infected during the study through their risk behavior.

4.3.6.4. Lessons learned relevant to regulatory evaluation. In the end, many lessons were learned from the conduct of HVTN 505. One lesson is that one must be prepared to adjust the study design of an on-going study when the results of other studies become available, e.g., when PrEP became more widely used in the U.S., where HVTN 505 was being conducted. Although the rate of PrEP uptake was not high, there needed to be a means to assess and monitor the uptake and adjust the study so that it did not become underpowered to assess efficacy as a consequence of other prevention strategies being used in the study communities. In fact, PrEP uptake during the study varied by location of site from as little as about 2% to as much as 12%. This likely reflects differences in trends among various HIV-affected communities in the U.S. Regulators need to be aware of the prevention strategies that may be considered to be standard-of-prevention in their country, communities and/or study populations in order to assure that trial sponsors are conducting their studies appropriately. Further, regulators need to consider the review and evaluation of study protocol amendments when new prevention strategies begin to be implemented. While it is generally considered to be inappropriate to amend primary endpoints of efficacy studies that are on-going, other events that have occurred locally or globally may require such amendments be considered in order to maintain appropriate ethical standards while assuring that a study continues to have the possibility to meet its stated aims.

4.3.7. NIH Mini-Summit on Adenovirus-vectored HIV vaccines

Given the results of STEP, Phambili, and HVTN 505, a Mini-Summit was held on 19 September 2013, hosted by NIH, who had funded these three studies. The Mini-Summit brought together experts to address certain questions and uncertainties arising from the outcomes of these studies. Although it was obvious from the study results that the tested Ad5-based HIV vaccines were not efficacious, it is unclear as to what impact the results from these vaccines have on candidates based on alternative adenovirus-based vectors. Both alternative (lower seroprevalent) human adenoviruses, as well as other species' adenoviruses, are being explored in preclinical and early clinical studies. While they are all members of a particular family of viruses (adenoviruses), meaning they are genetically related and share many commonalities, their biologies can be very different, including receptor usage, organ and species tropism, epidemiology, clinical syndromes they cause, etc. In addition, while the STEP study had a safety signal (enhanced acquisition), which was observed in a subset of vaccinated individuals, this safety signal was not clearly seen in Phambili (which was halted before full enrollment) or HVTN 505 studies.

The major conclusion of the Mini-Summit was that the Merck Ad5 HIV vaccine candidate, which was based on Ad5 and did not express Env, was associated with enhanced risk of HIV acquisition, but that everything else was uncertain. The VRC Ad5 HIV vaccine candidate was not associated with enhanced risk of HIV acquisition. The VRC Ad5 vaccine candidate differs in significant ways from the Merck Ad5 HIV vaccine candidate, including the expression of Env, which elicits antibody responses as well as T cell responses. The differences between the candidates are outlined in Section 4.3.6 above. Despite the uncertainties, it was concluded that there is sufficient scientific justification to continue to investigate alternative adenovirus serotypes and other species' adenoviruses as vectors of HIV vaccine candidates, including in heterologous prime-boost combinations. Exploration of the biological mechanism of enhancement of infection, as well as a better understanding of the biology of the vectors, must be a scientific priority, to ensure safe development of future HIV vaccine candidates, whether they are based on adenovirus vectors or other vectors. Understanding the mucosal immune response to vaccines and vectors, particularly activation of T cells and homing of T

Table 1
Summary of lessons learned from completed HIV/AIDS vaccine efficacy trials relevant to regulatory evaluation of clinical trial applications for vaccines in development.

Trial	Lessons learned relevant to regulatory evaluation
VAX004	• Need for clarity in statistical analysis plan to support clinical claims ◦ Sub-group analyses ◦ Hypothesis-generating vs. hypothesis-testing ◦ Statistical adjustments for multiplicity
VAX003	• Studies in marginalized populations can be feasibly and successfully conducted • Needs political will & commitment on part of investigators, regulators, governments, participants
Step	• T cell immunity alone is unlikely to be sufficient to prevent HIV infection or modulate disease in vaccinees who become infected through risk behavior ◦ Regulators should expect applicant to explain intended mode-of-action of vaccine & it should include some element of antibody-mediated immunity (T cell immunity may be necessary but insufficient) • Despite scientific hypotheses, it remains unknown what type(s) or levels of immunity are required for a successful HIV/AIDS vaccine, so applicant's justification should be reviewed with an agnostic perspective as to strength of justification and plausibility • Vaccine-elicited immune responses may paradoxically enhance risk of HIV infection instead of protecting against it ◦ This potential risk should be described in informed consent process & documents • Need for clarity in statistical analysis plan: ◦ One-tailed vs. two-tailed (vaccine could be worse or better than control) p-values ◦ Associations of efficacy endpoints & sub-group risk prognostic factors
Phambili	• T cell immunity alone is unlikely to be sufficient to prevent HIV infection or modulate disease in vaccinees who become infected through risk behavior • Balance value of continued blinded safety follow-up in trial halted early vs. ethics of informing participants by unblinding when trial is halted except for continued safety follow-up
RV144	• An HIV/AIDS vaccine can prevent acquisition of HIV infection • Need for scientific openness (remain agnostic) when reviewing applicant's proposed hypotheses & mode-of-action ◦ Only a human efficacy trial that is well-designed, well-controlled, & well-conducted can provide necessary data on whether a proposed vaccine regimen can protect humans against HIV acquisition • Need to consider the impact of outcomes of efficacy trials of components or related products to the regimen/vaccine under review or while the reviewed trial is on-going ◦ Because a component tested alone or a related product or regimen fails to have efficacy does not necessarily mean that the regimen being tested will also fail (the sum may be greater than the parts) • Statistical plan: ◦ For preventive vaccines, MITT analysis may be most meaningful ◦ Regulators should receive for review prospectively defined statistical analysis plan & possible interpretations/potential label claims to be made ◦ For credibility & regulatory decision-making, agreement should be gained in advance on what would constitute a type (which endpoint) and amount of efficacy that would be considered to support licensure, including lower bounds of 95% confidence intervals (e.g., they should exceed what amount in order to provide credible, compelling evidence sufficient to warrant licensure) ◦ Multiplicity of immunological endpoints, need for prioritization of statistical analyses to retain credibility in outcomes • Impact of potential waning immunity on trial design, sample size, length of follow-up • Prospective plan for collecting "right" specimens to aid correlates analysis • Regulatory recognition of the distinction between pilot and pivotal efficacy trials in terms of design & statistical power, hypothesis-generation vs. hypothesis-testing • Different vaccine regimens/candidates may have different immune correlates of protection determined by their unique mode-of-action
HVTN 505	• Studies may need to be adjusted while on-going to account for outcomes in other trials or changes in standards of care &/or prevention ◦ Need to continue to perform studies meeting international ethical principles, even if practices change while study is on-going • Such adjustments may include changes to primary endpoint or to other endpoints, study procedures, sample size, etc.
All	• HIV/AIDS vaccine efficacy trials are complex, but can be successfully & ethically conducted giving credible results as to a candidate vaccine's/regimen's efficacy or lack thereof • Need for evaluating trials with scientific openness, recognizing uncertainties • Some HIV/AIDS vaccine candidates can be protective against HIV infections acquired through risky behaviors, but others can paradoxically enhance risk of HIV infections acquired through risky behaviors • Type & amount of immune response from HIV/AIDS vaccine candidates needed for efficacy remains unknown, but will likely require at least some type of antibody-mediated effect • Clarity on statistical analysis plans is essential

cells that may be susceptible to HIV infection to mucosal sites following exposure to vectors or vaccines is also crucial. The advice by experts given to the NIH during the Mini-Summit will guide future funding decisions by the NIH and likely, other donors [20].

5. Conclusions

The clinical trials that have been conducted to date have not yet revealed a highly efficacious HIV/AIDS vaccine candidate or regimen. However, innumerable lessons have already been learned from the conduct of these studies and this review highlights those lessons learned as relevant to the regulatory considerations to be taken upon review of a CTA. A tabular summary of these is provided in Table 1. This review along with the companion review should aid regulators worldwide to focus on the key aspects and scientific uncertainties that present regulatory hurdles to their decision-making. However, regulators in individual countries, reviewing a CTA, should not feel that they are alone in this matter. As will be discussed in greater detail in the companion review and as was introduced in this review, advice from various WHO-supported regulatory fora can and should be sought to aid in dealing with these scientific uncertainties when making regulatory decisions.

Among the key lessons learned in the conduct of the six completed well-designed, well-conducted efficacy studies is that HIV/AIDS vaccine efficacy studies can be conducted safely and ethically, with good recruitment and retention, and giving definitive and useful results. Although each of the six completed trials had some degree of controversy associated with it, these controversies arose as a result of scientific uncertainty and not for reasons of misconduct or poor design. Each subsequent study built on

lessons learned from the previous one(s). It is clear from this review that those involved with HIV vaccine trials should remain agnostic and humble in the considerations of the merit of future clinical trials, including when candidates should advance to efficacy studies. Despite what may be the most prevailing scientific theory at any given moment, efficacy trials should continue and be designed sufficiently to give definitive results, upon which regulatory decisions may be made, when appropriate. It is unlikely that vaccines that work by simply eliciting a single arm of the immune system will protect against HIV acquisition or viral load control in those who become infected despite vaccination. Correlates analyses should be planned prospectively hand-in-hand with efficacy study trial design and the appropriate specimens, in regards to timing of collection, handling, and storage, must be taken. Likewise, appropriate assay methods need to be used, to facilitate identification and/or characterization of potential immune correlates of protection. Similarly, appropriate immunological samples may help to characterize untoward safety signals should they be seen in future efficacy trials.

This review has shown that statistical plans must be clear in many regards. Not only should decisions prospectively be made about which analyses should proceed as hypothesis-testing or only as hypothesis-generating when primary analyses fail to achieve significance, requiring a hierarchical arrangement of analyses, but also that clarity is required regarding the relevance of per-protocol, intent-to-treat, and modified-intent-to-treat analyses. This is especially true when they do not all reach the same statistical significance (though trends to significance may be concordant with other significant outcomes). The review also confirms that HIV acquisition must be part of the primary analysis of an HIV/AIDS vaccine efficacy trial, because it is known that prevention of acquisition by vaccination is feasible. A communications plan prospectively in place before the results of a study becomes known is crucial to fair and balanced reporting of results without raising false hopes or confusion and thus, discord, controversy, or distrust. Credibility is key to the ability to make regulatory decisions.

We have learned that populations that may have thought to be impossible to recruit and retain can be studied successfully through the political will of the researchers, communities, governments, and countries in which those studies are to take place. It is also known that the informed consent process must continue to make potential participants aware that the experimental vaccine(s) they may or may not receive in a controlled study may not only not benefit them, but could be harmful to them. Although the hope is that any vaccine candidate will be of benefit, of course, those involved should be agnostic until the study is completed and before there is evidence to know whether the vaccine candidate was protective, was not protective but not harmful, or was harmful. Regulators need to continue ensuring trials are planned with appropriate informed consent processes. Similarly, regulators need to be cognizant of the status of other prevention modalities and standard of care and treatment in use in their country and the communities where potential efficacy trials will take place in order to ensure that sponsors address these issues in their trial design and planning.

Achieving the development of a safe, effective, globally-useful HIV/AIDS vaccine is paramount to the control of the epidemic and regulators should expect they will need to make decisions in the face of and despite significant scientific uncertainty. Regulators in individual countries are not alone in facing their decisions when reviewing CTAs and should recognize the resources available to them, particularly from the World Health Organization. It is hoped that this and the companion review will aid regulators in LMIC feel more prepared for this challenge.

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